ABSTRACT:

Liquid-liquid phase separation has recently been recognized as a new principle by which membrane-less intracellular compartments are formed.[1-5] These cellular bodies are composed of non-stoichiometric assemblies of thousands of different protein and nucleic acids which segregate themselves from the surrounding cytoplasm to form complex coacervates. Intrinsically disordered multivalent proteins with low complexity domains have been found to be the drivers of phase separation.[6-11] The intrinsically disordered regions (IDRs) act as highly dynamic linkers and promote "fuzzy" interactions between the repetitive interaction domains in a variety of combinations.[12] However, the molecular origin of liquid-liquid phase separation remains unknown. Here, we demonstrate the change in the conformational landscape and the associated chain dynamics of an intrinsically disordered protein, tau k18, in the protein-rich de-mixed phase, utilizing an array of biophysical tools. Using an intramolecular proximity readout, we show that tau k18 undergoes conformational expansion which enables it to form intermolecular cross-talks between the polypeptide chains upon phase separation. We also demonstrate that these phase-separated proteinaceous droplets are not water excluded and the polypeptide chains experience significant chain solvation with the help of Stern-Volmer quenching experiments. Using time-resolved fluorescence anisotropy measurements we reveal that the polypeptide chain dynamics increases inside the protein droplets indicating rapid conformational fluctuations which enable weak, transient intermolecular interactions between the "sticky" domains of the polypeptide chains. Such polypeptide chain fluctuations are of crucial importance as they help in maintaining the liquid-like nature of the condensates. In summary, our results, together with conformational expansion and extensive conformational dynamics during phase separation facilitate transient intermolecular interaction, thus promoting liquid-liquid phase separation.